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Hydroxyproline-Rich Protein Material in Wood and Lignin of *Fagus sylvatica*

INGRID DILL, JOHANN SALNIKOW, AND GUNDA KRAEPELIN*

Institut für Biochemie und Molekulare Biologie, Abteilung Botanik, Technische Universität Berlin, D-1000 Berlin 10, Federal Republic of Germany

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The nitrogen content, distribution, and amino acid composition of protein material were determined in wood and lignin of *Fagus sylvatica*. The data indicated that the nitrogen originated from hydroxyproline-rich cell wall glycoprotein, about half of which may be bound to the lignin polymer. The implications for lignocellulose biodegradation are discussed.

It is well known that lignin preparations from wood may contain appreciable quantities of nitrogen. It has been pointed out that this nitrogen content may result from protein contamination, depending on the method used to prepare insoluble lignin (1). Such contamination could arise by some kind of nonspecific adsorption or condensation reaction between plant proteins and the lignin polymer, known to have a remarkable affinity for nitrogen compounds (9). Results of this type were mostly obtained from nonwoody tissues with a high nitrogen content. On the other hand, Whitmore (10) clearly demonstrated that lignin preparations (Klason lignin) from callus cultures of *Pinus elliottii* contained a hydroxyproline-rich protein, presumably derived from cell wall glycoproteins.

In the course of our studies on the biodegradation of hardwood, a substrate extremely lacking in nitrogen, we also determined the content, distribution, and chemical nature of the nitrogen in nondegraded wood samples and in Klason lignin preparations from European beech. Our results not only confirm that protein constitutes the nitrogen source in wood but may also contribute to an understanding of its regulatory role in the biodegradation of the lignin component in wood.

Wood samples were dried (105°C) and ground to pass through a 60-mesh screen. Before Klason lignin determinations were made, the wood was extracted with ethanol-benzene (1:2 [vol/vol]) for 4 h in a Soxhlet extractor. Klason lignin was determined by a modification of the TAPPI standard method (T 222os-74). Flasks containing 1 g of wood meal and 20 ml of H_2SO_4 (72%) were gently shaken in a water bath at 30°C for 1 h. The acid was then diluted with H_2O to 4% (wt/vol), and the samples were autoclaved at 121°C for 30 min. The lignin that settled overnight was collected by filtration through a crucible and washed free of acid with hot water. Before it was used for nitrogen determinations and HCl hydrolysis, the dried (105°C) lignin was thoroughly ground in a porcelain mortar. Nitrogen determinations were made by the Kjeldahl method, starting with 2 g of dry wood meal or 0.6 to 0.8 g of lignin. Titration was performed with 0.01 N H_2SO_4, and the pH was controlled potentiometrically.

For amino acid analysis, carefully weighed samples of wood and lignin containing adequate amounts of norleucine as an internal standard were hydrolyzed with 5.7 N HCl in sealed glass ampoules at 105°C for 20 to 120 h. The samples were dried, and the residues were dissolved in 0.2 N sodium citrate buffer (pH 2.2) for amino acid analysis; insoluble particles were removed by centrifugation. Aliquots of the samples were analyzed on a Durrum D-500 amino acid analyzer.

Table 1 shows amino acid compositions after the hydrolysis of wood and Klason lignin from *Fagus sylvatica*. To exclude the possibility that exogenous free amino acids could somehow become incorporated into the lignin fraction during preparation, we added norleucine to a parallel sample before extracting lignin. As expected, this amino acid was practically absent in the lignin fraction obtained by the standard method, indicating that the observed amino acids stemmed from a lignin-specific constituent. When the time of hydrolysis was varied from 20 h up to 120 h, a distinctive increase in the amounts of valine and isoleucine, characteristic for hydrophobic peptide bonds, was noted. On the basis of these results we conclude that the observed amino acid pattern reflects protein material genuinely present in the lignin component.

The rather high hydroxyproline content in hydrolysates of wood and of the insoluble lignin fraction suggests that the protein material in question actually was, or at least contained, hydroxyproline-rich cell wall proteins or glycoproteins (3, 5). Moreover, the amino acid patterns were very similar to each other, although more detailed studies with isolated lignoproteins are needed to substantiate their relationship.

Table 2 shows the results of Kjeldahl determinations of nitrogen content in parallel samples of wood and lignin. A comparison of the values with the nitrogen concentration calculated from the amino acid analysis (included in Table 2) makes clear that in both cases, the nitrogen content stemmed almost exclusively from amino acid nitrogen. It is remarkable that the nitrogen concentration on a dry-weight basis was more than two times higher in the lignin fraction than in the corresponding wood sample. An analogous distribution of nitrogen was found in other hardwoods, e.g., Chilean *Eucryphia cordifolia* and *Nothofagus dombeyi*, as well as in an additional sample from another *F. sylvatica* tree. One explanation for this could be that cell wall proteins were artificially enriched in the lignin fraction during preparation. On the other hand, calculation of the distribution of nitrogen between the lignin- and nonlignin constituents revealed that the lignin fraction always contained at least 50% of the total nitrogen, irrespective of the individual amounts of lignin and nitrogen in the wood (Table 2).

It is generally accepted that hydroxyproline-rich glycoproteins are integral components of the cell walls of higher

* Corresponding author.

TABLE 1. Amino acid compositions of hydrolysates of wood and lignin from *F. sylvatica*[a]

Amino acid	Amino acid composition			
	Wood		Lignin	
	nmol of amino acid per mg	Mol of amino acid per 100 mol	nmol of amino acid per mg	Mol of amino acid per 100 mol
Asp	9.85	10.11	20.62	8.52
Thr	6.09	6.26	14.34	5.92
Ser[b]	6.81	6.99	14.27	5.89
Glu	10.39	10.67	28.34	11.71
Pro	9.52	9.78	25.34	10.47
Gly	9.66	9.92	22.34	9.23
Ala	8.55	8.78	20.89	8.63
Val	6.67	6.85	18.96	7.83
Met	0.22	0.23	0.45	0.19
Ile	4.61	4.74	13.20	5.45
Leu	7.53	7.73	20.72	8.56
Tyr[b]	0.72	0.74	1.19	0.49
Phe	3.42	3.51	5.82	2.40
His	1.88	1.93	6.95	2.87
Lys	3.06	3.14	7.15	2.95
Arg	1.62	1.66	6.95	2.87
Hyp	6.76	6.94	14.58	6.02

[a] Data are the averages of duplicate determinations. Hydrolysis was carried out for 120 h, except as otherwise noted.
[b] Hydrolysis was carried out for 20 h.

TABLE 2. Nitrogen content of wood and lignin[a]

Species	Klason lignin (% dry wt)	Testing method	Nitrogen (μg/mg) in:		% Nitrogen in lignin
			Wood	Lignin	
Fagus sylvatica I	25	Kjeldahl	1.65	3.76	57.0
		Amino acid analysis	1.53	3.98	65.0
Fagus sylvatica II	21	Kjeldahl	0.90	2.20	51.3
Eucryphia cordifolia	28	Kjeldahl	0.58	1.12	54.1
Nothofagus dombeyi	17	Kjeldahl	0.42	1.26	51.0

[a] Data are the averages of duplicate determinations.

plants. The middle lamella and the primary wall apparently contain the highest amounts of glycoproteins, and these parts of the cell wall later become the most lignified ones (8). Whitmore (10) suggested that the polymerizing lignin is covalently linked to cell wall glycoproteins and that these bonds may preferentially be formed with hydroxyproline. If this also applies to our wood samples, the consistently higher nitrogen values found in the insoluble lignin component could be explained by the previous removal of the larger, but nitrogen-poor, nonlignin portion of the cell walls. The results obtained would then correspond to real differences in nitrogen concentrations within the cell walls of wood.

It is a well-known phenomenon that nitrogen supply plays a crucial regulatory role in the biodegradation of lignin components in wood. Thus, under laboratory conditions, nitrogen starvation is the most effective parameter for inducing secondary metabolism and ligninolytic activity in the white rot fungus *Phanerochaete chrysosporium* (4, 6, 7). This means that the ligninolytic enzyme system, in this case, becomes nonspecifically activated when the fungus enters the stationary phase of growth, whereas lignin by itself does not induce ligninolytic activity. Moreover, a pleiotropic mutant of *P. chrysosporium* lacking several properties associated with the onset of secondary metabolism (phenol oxidase, production of veratryl alcohol, and fruit body formation) also turned out to be defective in lignin degradation (2).

The question arises as to whether data obtained with laboratory cultures of *P. chrysosporium* also apply to other white rot fungi and, in particular, to wood degradation under field conditions. We believe that our results could contribute to a better understanding of the ecological situation. If we take into account that about half of the extremely limited nitrogen source in wood may be firmly linked to the lignin polymer as a lignoprotein complex, then a white rot fungus should be able to gain sufficient nitrogen for its growth and development in only two ways: degradation of lignin and recycling of nitrogen from older parts of the mycelium. In any case, the low concentration of available nitrogen in wood does not allow the sequence of extensive primary growth followed by stationary-phase metabolism that is usually found in laboratory batch cultures. Therefore, we suggest that under field conditions, the fungus permanently displays a "quasi-stationary" metabolism, with ligninolytic activity being expressed either permanently or stepwise. In the latter case, degradation of lignin would yield the nitrogen needed for a limited degradation of carbohydrates and a period of restricted growth during which ligninolytic activity may be repressed. After the available nitrogen is exhausted, growth stops and lignin degradation starts again. The results of Ulmer et al. (7) showing intermittent growth cycles in *P. chrysosporium* under nitrogen-limited chemostat conditions may support our suggestion. Finally, it would be interesting to find out whether brown rot fungi, in contrast to white rot fungi, have developed a mechanism for extracting nitrogen from the lignin complex, as they extensively metabolize the carbohydrate components of wood but apparently do not require a simultaneous degradation of lignin. Further studies regarding these questions are in progress.

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